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=> s CD39
L1         1106 CD39

=> s l1 and human
L2          712 L1 AND HUMAN

=> s l2 and "CD39L4"
L3           17 L2 AND "CD39L4"

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=> s l3 and modification
L5            0 L3 AND MODIFICATION

=> dup remove l17
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L6             7 DUP REMOVE L3 (10 DUPLICATES REMOVED)

=> d l6 1-7 cbib abs

L6   ANSWER 1 OF 7  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
2003409979  EMBASE  Bacterial expression, characterization, and disulfide bond
     determination of soluble human NTPDase6 (CD39L2) nucleotidase:
     Implications for structure and function. Ivanenkov V.V.; Murphy-Piedmonte
     D.M.; Kirley T.L.. T.L. Kirley, Dept. of Pharmacol./Cell Biophys., College
     of Medicine, University of Cincinnati, P.O. Box 670575, Cincinnati, OH
     45267-0575, United States. terry.kirley@uc.edu. Biochemistry  42/40
     (11726-11735)  14 Oct 2003.
     Refs: 43.
     ISSN: 0006-2960. CODEN: BICHAW. Pub. Country: United States. Language:
     English. Summary Language: English.
AB   The ectonucleoside triphosphate diphosphohydrolases (NTPDases) control
     extracellular nucleotide concentrations, thereby modulating many important
     biological responses, including blood clotting and pain perception.
     NTPDases1-4 are oligomeric integral membrane proteins, whereas NTPDase5 (
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CD39L4) and NTPDase6 (CD39L2) are soluble monomeric enzymes, making them more amenable to thorough structural and functional analyses than the membrane-bound forms. Therefore, we report here the bacterial expression, refolding, purification, and biochemical characterization of the soluble portion of **human** NTPDase6. Consistent with the enzyme expressed in mammalian cells, this recombinant NTPDase6 efficiently hydrolyzes GDP, IDP, and UDP (specific activity of approximately 50000, .mu.mol mg(-1) h(-1)), with slower hydrolysis of CDP, ITP, GTP, CTP, ADP, and UTP and virtually no hydrolysis of ATP. The K(m) for GDP (130 .+-. 30 .mu.M) is similar to that determined for the soluble rat NTPDase6 expressed in mammalian cells. The secondary structure of the refolded enzyme was determined by circular dichroism to be 33% .alpha.-helix, 18% .beta.-sheet, and 49% random coil, consistent with the secondary structure predicted from the amino acid sequence of soluble NTPDase6. Four of the five cysteine residues in the soluble NTPDase6 are highly conserved among all the NTPDases, while the fifth residue is not. Mutation of this nonconserved cysteine resulted in an enzyme very similar to wild type in its enzymology and secondary structure, indicating that this cysteine exists as a free sulfhydryl and is not essential for structure or function. The disulfide pairing of the other four cysteine residues was determined as Cys (249)-Cys(28) and Cys(340)-Cys(354) by HPLC and mass spectral analysis of tryptic peptides. Due to conservation of these cysteine residues, these two disulfide bonds are likely to exist in all NTPDases. A structural model for NTPDase6, incorporating these and other findings obtained with other NTPDases, is proposed.

L6 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
2002:151472 Document No. 136:215415 **CD39**-like polypeptides and nucleic acids for diagnosis and therapy. Chadwick, Brian Paul; Frischauf, Anna-Maria (Hyseq, Inc., USA). U.S. US 6350447 B1 20020226, 101 pp. (English). CODEN: USXXAM. APPLICATION: US 1999-240639 19990129.
AB The present invention provides **CD39**-like polynucleotides and proteins encoded by such polynucleotides. The **CD39**-like polypeptides, agonists and antagonists, encoding polynucleotides, oligonucleotide probes and primers, and antibodies are useful as and for screening immunostimulant or immunosuppressant, for diagnosing and treating hematopoietic, immunol., infectious, or autoimmune diseases. The polypeptides and polynucleotides may also useful as research marker (chromosome marker and mol. wt. marker), gene mapping, gene chip, nutritional source and supplement, and others.

L6 ANSWER 3 OF 7 MEDLINE on STN DUPLICATE 1
2001667580 Document Number: 21570130. PubMed ID: 11713596. Identity between the PCPH proto-oncogene and the **CD39L4** (ENTPD5) ectonucleoside triphosphate diphosphohydrolase gene. Paez J G; Recio J A; Rouzaut A; Notario V. (Laboratory of Experimental Carcinogenesis, Department of Radiation Medicine, Georgetown University Medical Center, Washington, DC 20007, USA.) INTERNATIONAL JOURNAL OF ONCOLOGY, (2001 Dec) 19 (6) 1249-54. Journal code: 9306042. ISSN: 1019-6439. Pub. country: Greece. Language: English.
AB PCPH was initially defined as a proto-oncogene on the basis of its frequent detection as an activated oncogene in tumorigenic Syrian hamster embryo fibroblast cell lines converted to the neoplastic state by a single treatment with the carcinogen 3-methylcholanthrene (MC). Further studies identified the translation product of the PCPH gene as a ribonucleotide-binding protein with special affinity for ribonucleoside diphosphates. Later, we showed that the PCPH protein was homologous to the product of the yeast GDA1 gene and demonstrated that it had intrinsic guanosine diphosphatase activity, although it did not complement the disrupted phenotype when expressed in gda1 null Saccharomyces cerevisiae strains. These results indicated that the primary function of PCPH was unlikely to be related to the ribonucleotide recycling function that its yeast counterpart performs in the Golgi during the process of protein glycosylation. However, taken together, our data strongly suggested that the normal cellular function of PCPH was related to ribonucleotide

metabolism. We now report that PCPH is structurally and functionally identical to the mammalian ectonucleoside triphosphate diphosphohydrolase **CD39L4** (ENTPD5), recently described as a member of the lymphoid activation antigen (<cluster of differentiation>) **CD39** protein family. These results may help to establish the normal cellular function of the PCPH proto-oncogene product and its role in neoplastic development during carcinogenesis.

L6 ANSWER 4 OF 7 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2000397566 EMBASE Biochemical characterization of **CD39L4**. Mulero J.J.; Yeung G.; Nelken S.T.; Bright J.M.; McGowan D.W.; Ford J.E.. J.E. Ford, Hyseq Inc., 670 Almanor Ave., Sunnyvale, CA 94086, United States. ford@sbh.com. Biochemistry 39/42 (12924-12928) 24 Oct 2000. Refs: 18.
ISSN: 0006-2960. CODEN: BICHAW. Pub. Country: United States. Language: English. Summary Language: English.

AB Nucleotides are involved in regulating a number of important processes ranging from inflammation to platelet aggregation. Enzymes that can modulate levels of nucleotides in the blood therefore represent important regulatory components in these physiological systems. **CD39L4** is a soluble E-nucleoside triphosphate dephosphohydrolase (E-NTPDase) with specificity for nucleotide diphosphates (NDPs). In this study, stable mammalian and insect cell lines were generated expressing **CD39L4** protein to purify and characterize the recombinant protein. We demonstrate that recombinant **CD39L4** protein expressed in **human** embryonic carcinoma 293 cells is glycosylated by comparing the molecular masses before and after glycosidase treatment. Activity measurements of **CD39L4** isolated from tunicamycin-treated, transiently transfected COS-7 cells indicate that glycosylation is not required for full ADPase activity. Recombinant **human CD39L4** protein isolated from stable insect cells was glycosylated differently, but also demonstrated relative activity comparable to that of the mammalian protein. When denatured by SDS under nonreducing conditions, a fraction of the **CD39L4** protein migrates as a 110 kDa disulfide-linked dimer. We determined that the monomer is the most active form of **CD39L4** by measuring the activity of sucrose density gradient fractions of monomers and partially purified dimers. The physiological significance of the biochemical and enzymatic characterization is discussed.

L6 ANSWER 5 OF 7 MEDLINE on STN DUPLICATE 2

2000502205 Document Number: 20496770. PubMed ID: 11041856. CD39L2, a gene encoding a **human** nucleoside diphosphatase, predominantly expressed in the heart. Yeung G; Mulero J J; McGowan D W; Bajwa S S; Ford J E. (Functional Genomics Department, Immunology Group, Hyseq Inc., 670 Almanor Avenue, Sunnyvale, California 94086, USA.) BIOCHEMISTRY, (2000 Oct 24) 39 (42) 12916-23. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB E-NTPDases are extracellular enzymes that hydrolyze nucleotides. The **human** E-NTPDase gene family currently consists of five reported members (**CD39**, CD39L1, CD39L2, CD39L3, and **CD39L4**). Both membrane-bound and secreted family members have been predicted by encoded transmembrane and leader peptide motifs. In this report, we demonstrate that the **human** CD39L2 gene is expressed predominantly in the heart. In situ hybridization results from heart indicate that the CD39L2 message is expressed in muscle and capillary endothelial cells. We also show that the CD39L2 gene encodes an extracellular E-NTPDase. Flow cytometric experiments show that transiently expressed CD39L2 is present on the surface of COS-7 cells. Transfected cells also produce recombinant glycosylated protein in the medium, and this process can be blocked by brefeldin A, an inhibitor of the mammalian secretory pathway. The enzymology of CD39L2 shows characteristic features of a typical E-NTPDase, but with a much higher degree of specificity for NDPs over NTPs as enzymatic substrates. The kinetics of the ADPase activity exhibit positive cooperativity. The

predominance of CD39L2 expression in the heart supports a functional role in regulating platelet activation and recruitment in this organ.

L6 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
1999:466987 Document No. 131:239542 **CD39**-L4 is a secreted **human** apyrase, specific for the hydrolysis of nucleoside diphosphates. Mulero, Julio J.; Yeung, George; Nelken, Sarah T.; Ford, John E. (Functional Genomics Department, Hyseq Inc., Sunnyvale, CA, 94086, USA). Journal of Biological Chemistry, 274(29), 20064-20067 (English) 1999. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB The **human** ecto-apyrase gene family consists of five reported members (**CD39**, **CD39**-L1, **CD39**-L2, **CD39**-L3, and **CD39**-L4). The family can be subdivided into two groups by conservation of proposed structural domains. The **CD39**, **CD39**-L1, and **CD39**-L3 genes all encode hydrophobic portions in their carboxy and amino termini, serving as transmembrane domains for **CD39** and potentially for the other two members. **CD39**-L2 and **CD39**-L4 genes encode hydrophobic portions in their amino termini, suggesting that they might encode secreted apyrases. We demonstrate that the **CD39**-L4 gene encodes the first reported **human** secreted ecto-apyrase. COS-7 cells transfected with a **CD39**-L4 expression construct utilizing the naturally occurring leader peptide express recombinant protein outside of the cells. This expression can be blocked by brefeldin A, a chem. that inhibits a step in mammalian secretory pathways. We also demonstrate expression of **CD39**-L4 message in macrophages, suggesting that the protein is present in the circulation. Furthermore, we show that **CD39**-L4 is an E-type apyrase, is dependent on calcium and magnesium cations, and has high degree of specificity for NDPs over NTPs as enzymic substrates. A potential physiol. role in hemostasis and platelet aggregation is presented.

L6 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 3
1998341119 Document Number: 98341119. PubMed ID: 9676430. The **CD39**-like gene family: identification of three new **human** members (CD39L2, CD39L3, and **CD39L4**), their murine homologues, and a member of the gene family from Drosophila melanogaster. Chadwick B P; Frischauf A M. (Imperial Cancer Research Fund, London, United Kingdom.) GENOMICS, (1998 Jun 15) 50 (3) 357-67. Journal code: 8800135. ISSN: 0888-7543. Pub. country: United States. Language: English.

AB The **human** lymphoid cell activation antigen **CD39** is a known E-type apyrase that hydrolyzes extracellular ATP and ADP, a function important in homotypic adhesion, platelet aggregation, and removal by activated lymphocytes of the lytic effect of ATP. The recently identified putative rat homologue of CD39L1 has been shown to have E-type ecto-ATPase activity, by hydrolyzing extracellular ATP. We have characterized three novel **CD39**-like transcripts, CD39L2, CD39L3, and **CD39L4**, which share extensive amino acid homology with other nucleotide triphosphatases in vertebrates, invertebrates, and plants, suggesting that these genes also encode proteins with ecto-nucleotidase activity. Isolation and sequencing of full-length cDNA clones for each gene identified putative proteins of 485, 529, and 429 amino acids. The expression pattern of all five **human** members of the gene family was analyzed. CD39L2, CD39L3, and **CD39L4** were mapped on the **human** genome, and the murine homologues identified with the putative map locations were assigned on the basis of regions of conserved gene order between **human** and mouse chromosomes. The map location of mcd3914 places the gene within a region associated with audiogenic seizure susceptibility in mouse. This disorder is characterized by convulsions induced by loud high-frequency sound and has been shown to be associated with increased nucleotide triphosphatase activity.

=> s (ford j?/au or mulero j?/au)
L7 6979 (FORD J?/AU OR MULERO J?/AU)

=> s l7 and human CD39
L8 11 L7 AND HUMAN CD39

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PROCESSING COMPLETED FOR L8
L9 7 DUP REMOVE L8 (4 DUPLICATES REMOVED)

=> d l9 1-7 cbib abs

L9 ANSWER 1 OF 7 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
2003:32122 Document No.: PREV200300032122. Methods and materials relating to CD39-like polypeptides. **Ford, John** [Inventor, Reprint Author]; **Mulero, Julio J.** [Inventor]; Yeung, George [Inventor]. ASSIGNEE: Hyseq, Inc.. Patent Info.: US 6476211 November 05, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (Nov 5 2002) Vol. 1264, No. 1. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133 (ISSN print). Language: English.
AB The invention provides polynucleotides isolated from cDNA libraries of human fetal liver-spleen and macrophage as well as polypeptides encoded by these polynucleotides and mutants or variants thereof. The polypeptides correspond to a **human CD39**-like protein. Other aspects of the invention include vectors containing polynucleotides of the invention and related host cells as well a processes for producing CD39-like polypeptides, and antibodies specific for such polypeptides.

L9 ANSWER 2 OF 7 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2
2002:582154 Document No.: PREV200200582154. Methods and materials relating to novel CD39-like polypeptides. **Ford, John** [Inventor]; **Mulero, Julio J.** [Inventor]; Yeung, George [Inventor, Reprint author]. San Mateo, CA, USA. ASSIGNEE: Hyseq, Inc.. Patent Info.: US 6447771 September 10, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (Sep. 10, 2002) Vol. 1262, No. 2. http://www.uspto.gov/web/menu/patdata.html. e-file. CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB The invention provides polynucleotides isolated from cDNA libraries of human fetal liver-spleen and macrophage as well as polypeptides encoded by these polynucleotides and mutants or variants thereof. The polypeptides correspond to a **human CD39**-like protein. Other aspects of the invention include vectors containing polynucleotides of the invention and related host cells as well a processes for producing CD39-like polypeptides, and antibodies specific for such polypeptides.

L9 ANSWER 3 OF 7 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3
2002:337543 Document No.: PREV200200337543. Methods and materials relating to novel CD39-like polypeptides. **Ford, John E.** [Inventor, Reprint author]; **Mulero, Julio J.** [Inventor]. San Mateo, CA, USA. ASSIGNEE: Hyseq, Inc.. Patent Info.: US 6387645 May 14, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (May 14, 2002) Vol. 1258, No. 2. http://www.uspto.gov/web/menu/patdata.html. e-file. CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB The invention provides novel polynucleotides isolated from cDNA libraries of human fetal liver-spleen and macrophage as well as polypeptides encoded by these polynucleotides and mutants or variants thereof. The polypeptides correspond to a novel **human CD39**-like protein. Other aspects of the invention include vectors containing polynucleotides of the invention and related host cells as well a processes for producing novel CD39-like polypeptides, and antibodies specific for such polypeptides.

L9 ANSWER 4 OF 7 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4
2002:144596 Document No.: PREV200200144596. Methods and materials relating to CD39-like polypeptides. **Ford, John** [Inventor]; **Mulero, Julio J.** [Inventor, Reprint author]; Yeung, George [Inventor]. Palo Alto, CA, USA. ASSIGNEE: Hyseq, Inc.. Patent Info.: US 6335013 January 01, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (Jan. 1, 2002) Vol. 1254, No. 1. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB The invention provides novel polynucleotides isolated from cDNA libraries of human fetal liver-spleen and macrophage as well as polypeptides encoded by these polynucleotides and mutants or variants thereof. The polypeptides correspond to a novel **human CD39**-like protein. Other aspects of the invention include vectors containing polynucleotides of the invention and related host cells as well a processes for producing novel CD39-like polypeptides, and antibodies specific for such polypeptides.

L9 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
2001:114897 Document No. 134:174556 **Human CD39**-like proteins and cDNAs and methods for drug screening and antithrombosis therapy. **Ford, John**; **Mulero, Julio J.**; Yeung, George (Hyseq Inc., USA). PCT Int. Appl. WO 2001010205 A1 20010215, 203 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US21790 20000809. PRIORITY: US 1999-370265 19990809; US 2000-481238 20000111; US 2000-557800 20000425; US 2000-583231 20000526; US 2000-608285 20000630.
AB The invention provides **human CD39**-like proteins and their cDNAs. The CD39-L2, CD39-L4, and CD39-L66 proteins possess apyrase activity. Other aspects of the invention include vectors contg. DNA of the invention, recombinant host cells expressing the DNA, processes for producing the CD39-like proteins, and antibodies specific for the proteins. Also disclosed are use of the CD39-like proteins as antithrombotics and for screening for modulators of the function of the CD39-like proteins. Thus, the cDNAs for human apyrases designated CD39-L2, CD39-L4, and CD39-L66 were cloned, sequenced, and expressed in COS7, 293 and insect cells, and their apyrase activity demonstrated. These appear to be a new class of E-type apyrase with a specificity for NDPs as substrates. Site-specific mutants of CD39-L4 were prepd. with enhanced apyrase activity. The human and mouse genes were also cloned and the gene for CD39-L4 was mapped to human chromosome 11.

L9 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
2000:68476 Document No. 132:135506 Identification of novel homologs of CD39 antigens of human and cDNAs encoding them. **Ford, John**; **Mulero, Julio** (Hyseq, Inc., USA). PCT Int. Appl. WO 2000004041 A2 20000127, 125 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US16180 19990716. PRIORITY: US 1998-118205 19980716; US 1998-122449 19980724; US 1999-244444 19990204; US 1999-273447 19990319; US 1999-350836 19990709.
AB CDNAs for homologs of the **human CD39** antigen are

cloned from cDNA libraries of human fetal liver-spleen and macrophage and the gene products are characterized. The proteins may be of use in the treatment of clotting disorders including thrombosis. Preliminary clones were obtained from a human fetal liver spleen cDNA library by detn. of a sequence signature sequence followed by sequencing of those clones with CD39-like signatures. The clone obtained encoded a CD39-like protein and hybridized to an mRNA from macrophages, but not from any other tissue tested. Unlike CD39, this protein was sol. and secreted from cells and was shown to be an apyrase. It also shared conserved sequences with other apyrases and mutation of the conserved regions affected the apyrase activity.

L9 ANSWER 7 OF 7 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1999:467417 Document No.: PREV199900467417. CD39-L4 is a secreted human apyrase, specific for the hydrolysis of nucleoside diphosphates. **Mulero, Julio J.**; Yeung, George; Nelken, Sarah T.; **Ford, John E.** [Reprint author]. Functional Genomics Dept., Hyseq Inc., 670 Almanor, Sunnyvale, CA, 94086, USA. Journal of Biological Chemistry, (July 16, 1999) Vol. 274, No. 29, pp. 20064-20067. print.
CODEN: JBCHA3. ISSN: 0021-9258. Language: English.
AB The human ecto-apyrase gene family consists of five reported members (CD39, CD39-L1, CD39-L2, CD39-L3, and CD39-L4). The family can be subdivided into two groups by conservation of proposed structural domains. The CD39, CD39-L1, and CD39-L3 genes all encode hydrophobic portions in their carboxy and amino termini, serving as transmembrane domains for CD39 and potentially for the other two members. CD39-L2 and CD39-L4 genes encode hydrophobic portions in their amino termini, suggesting that they might encode secreted apyrases. We demonstrate that the CD39-L4 gene encodes the first reported human secreted ecto-apyrase. COS-7 cells transfected with a CD39-L4 expression construct utilizing the naturally occurring leader peptide express recombinant protein outside of the cells. This expression can be blocked by brefeldin A, a chemical that inhibits a step in mammalian secretory pathways. We also demonstrate expression of CD39-L4 message in macrophages, suggesting that the protein is presentin the circulation. Furthermore, we show that CD39-L4 is an E-type apyrase, is dependent on calcium and magnesium cations, and has high degree of specificity for NDPs over NTPs as enzymatic substrates. A potential physiological role in hemostasis and platelet aggregation is presented.

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=> s l1 and NTPDase
L10         137 L1 AND NTPDASE

=> s l10 and ATP diphosphohydrolase
L11          39 L10 AND ATP DIPHOSPHOHYDROLASE

=> s l11 and ENTPD5
L12           0 L11 AND ENTPD5

=> dup remove l11
PROCESSING COMPLETED FOR L11
L13          27 DUP REMOVE L11 (12 DUPLICATES REMOVED)

=> d l13 1-27 cbib abs
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L13 ANSWER 1 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2004:40641 The Genuine Article (R) Number: 755RZ. Heterologous cell-cell interactions: thromboregulation, cerebroprotection and cardioprotection by **CD39** (**NTPDase**-1). Marcus A J (Reprint); Broekman M J; Drosopoulos J H F; Islam N; Pinsky D J; Sesti C; Levi R. Cornell Univ, Weill Med Coll, VA New York Healthcare Syst, 423 E 23rd St, New York, NY 10010 USA (Reprint); Cornell Univ, Weill Med Coll, VA New York Healthcare Syst, New York, NY 10010 USA; VA New York Harbor Healthcare Syst, Med Serv Hematol Oncol, New York, NY 10010 USA; VA New York Harbor Healthcare Syst, Res Serv Hematol Oncol, New York, NY 10010 USA; Columbia Univ, Coll Phys &

Surg, Dept Med, Div Cardiol, New York, NY USA; Columbia Univ, Coll Phys & Surg, Dept Med, Div Circulatory Physiol, New York, NY USA. JOURNAL OF THROMBOSIS AND HAEMOSTASIS (DEC 2003) Vol. 1, No. 12, pp. 2497-2509. Publisher: BLACKWELL PUBL LTD. 108 COWLEY RD, OXFORD OX4 1JF, OXON, ENGLAND. ISSN: 1538-7933. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Blood platelets maintain vascular integrity and promote primary and secondary hemostasis following interruption of vessel continuity. Biochemical or physical damage to the coronary, carotid or peripheral arteries is followed by excessive platelet activation and recruitment culminating in vascular occlusion and tissue ischemia. Currently inadequate therapeutic approaches to stroke and coronary artery disease are a public health issue. Following our demonstration of neutrophil leukotriene production from arachidonate released from activated aspirin-treated platelets, we studied interactions between platelets and other blood cells, leading to concepts of transcellular metabolism and thromboregulation. Thrombosis has a pro-inflammatory component whereby biologically active substances are synthesized by interactions between different cell types that could not individually synthesize the product(s). Endothelial cells control platelet reactivity via three biochemical systems-autacoids leading to production of prostacyclin and nitric oxide, and endothelial ecto-ADPase/**CD39**/**NTPDase** -1. The autacoids are fluid-phase reactants, not produced by tissues in the basal state. They are only synthesized intracellularly and released upon interactions of cells with an agonist. When released, autacoids exert fleeting actions in the immediate milieu, and are rapidly inactivated. **CD39** is an integral component of the endothelial cell surface and is substrate-activated. It maintains vascular fluidity in the complete absence of prostacyclin and nitric oxide, indicating that they are ancillary components of hemostasis. Therapeutic implications for the autacoids have not been compelling because of their transient, local and fleeting action, and limited potency. Conversely, **CD39**, acting solely on the platelet releasate, is efficacious in three different animal models. It metabolically neutralizes a prothrombotic platelet releasate via deletion of ADP-the major recruiting agent responsible for formation of an occlusive thrombus. In addition, solCD39 reduced ATP- and ischemia-induced norepinephrine release in the heart. This reduction can prevent fatal arrhythmia. Moreover, solCD39 ameliorated the sequelae of stroke in **CD39** null mice. **CD39** represents the next generation of cardioprotective and cerebroprotective molecules.

L13 ANSWER 2 OF 27 MEDLINE on STN DUPLICATE 1
2003378250 Document Number: 22782469. PubMed ID: 12899930. ATP and ADP hydrolysis in brain membranes of zebrafish (Danio rerio). Rico Eduardo Pacheco; Senger Mario Roberto; Fauth Maria da Graca; Dias Renato Dutra; Bogo Mauricio Reis; Bonan Carla Denise. (Departamento de Ciencias Fisiologicas, Faculdade de Biociencias, Pontificia Universidade Catolica do Rio Grande do Sul. Avenida Ipiranga 6681, 90619-900 RS, Porto Alegre, Brazil.) LIFE SCIENCES, (2003 Sep 5) 73 (16) 2071-82. Journal code: 0375521. ISSN: 0024-3205. Pub. country: England: United Kingdom. Language: English.

AB Nucleotides, e.g. ATP and ADP, are important signaling molecules, which elicit several biological responses. The degradation of nucleotides is catalyzed by a family of enzymes called **NTPDases** (nucleoside triphosphate diphosphohydrolases). The present study reports the enzymatic properties of a **NTPDase** (**CD39**, apyrase, **ATP diphosphohydrolase**) in brain membranes of zebrafish (Danio rerio). This enzyme was cation-dependent, with a maximal rate for ATP and ADP hydrolysis in a pH range of 7.5-8.0 in the presence of Ca(2+) (5 mM). The enzyme displayed a maximal activity for ATP and ADP hydrolysis at 37 degrees C. It was able to hydrolyze purine and pyrimidine nucleosides 5'-di and triphosphates, being insensitive to classical ATPase inhibitors, such as ouabain (1 mM), N-ethylmaleimide (0.1 mM), orthovanadate (0.1 mM) and sodium azide (0.1 mM). A significant inhibition of ATP and ADP hydrolysis (68% and 34%, respectively) was

observed in the presence of 20 mM sodium azide, used as a possible inhibitor of **ATP diphosphohydrolase**. Levamisole (1 mM) and tetramisole (1 mM), specific inhibitors of alkaline phosphatase and P1, P(5)-di (adenosine 5'-) pentaphosphate, an inhibitor of adenylate kinase did not alter the enzyme activity. The presence of a **NTPDase** in brain membranes of zebrafish may be important for the modulation of nucleotide and nucleoside levels, controlling their actions on specific purinoceptors in central nervous system of this specie.

L13 ANSWER 3 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:308928 The Genuine Article (R) Number: 665KH. Determination of native oligomeric state and substrate specificity of rat NTPDase1 and NTPDase2 after heterologous expression in Xenopus oocytes. Failer B U; Aschrafi A; Schmalzing G; Zimmermann H (Reprint). Univ Frankfurt, Biozentrum, AK Neurochem, Marie Curie Str 9, D-60439 Frankfurt, Germany (Reprint); Univ Frankfurt, Biozentrum, AK Neurochem, D-60439 Frankfurt, Germany; Univ Frankfurt Klinikum, Inst Allgemeine Pharmakol & Toxikol, D-6000 Frankfurt, Germany; Rhein Westfal TH Aachen, Inst Pharmakol & Toxikol, D-5100 Aachen, Germany. EUROPEAN JOURNAL OF BIOCHEMISTRY (APR 2003) Vol. 270, No. 8, pp. 1802-1809. Publisher: BLACKWELL PUBLISHING LTD. 9600 GARSINGTON RD, OXFORD OX4 2DG, OXON, ENGLAND. ISSN: 0014-2956. Pub. country: Germany. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB NTPDase1 and NTPDase2 are two related plasma membrane-located enzymes involved in the extracellular degradation of nucleoside 5'-tri- and -diphosphates. They differ regarding their hydrolysis ratios for ATP and ADP. Both enzymes have a predicted transmembrane domain close to the N- and C-terminus, respectively, connected by an extensive extracellular domain that carries the active site. We expressed the rat-derived enzymes in Xenopus laevis oocytes and analyzed their quarternary structure. As revealed by application of blue native PAGE and a comparison of glutaraldehyde cross-linking, native NTPDase1 and NTPDase2 occur in oligomeric form. Oligomer formation of the cell surface-located pool of the enzymes was verified by surface iodination. The two enzymes differed in oligomeric structure and in oligomer complex stability. NTPDase1 preferentially occurred as a dimer that could be dissociated into monomeric forms in the presence of Coomassie Brilliant blue G-250 and dithiothreitol whereas NTPDase2 revealed higher oligomeric forms up to tetramers, largely resistant to dithiothreitol. Our results further suggest that the enzymes exist in varying oligomeric states. In contrast to NTPDase1, substrate specificity of NTPDase2 was altered with prolonged expression time, resulting in a decrease in the ATPase/ADPase activity ratio from 10 : 1 to 2.5 : 1. This was accompanied by a transition into a higher oligomeric state. Our results suggest that despite close sequence identity, NTPDase1 and NTPDase2 differ in oligomeric structure. Dynamic alterations in oligomeric state may induce changes in substrate preference and thus influence the pattern of extracellular nucleotide degradation in situ.

L13 ANSWER 4 OF 27 MEDLINE on STN DUPLICATE 2
2003236544. PubMed ID: 12757773. Enzymes that hydrolyze adenine nucleotides in diabetes and associated pathologies. Lunkes Gilberto Inacio; Lunkes Daniela; Stefanello Francieli; Morsch Andre; Morsch Vera Maria; Mazzanti Cinthia Melazzo; Schetinger Maria Rosa Chitolina. (Departamento de Quimica, Centro de Ciencias Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil.) Thrombosis research, (2003 Feb 15) 109 (4) 189-94. Journal code: 0326377. ISSN: 0049-3848. Pub. country: United States. Language: English.
AB The activities of the enzymes **NTPDase** (E.C. 3.6.1.5, apyrase, **ATP diphosphohydrolase**, ecto-**CD39**) and 5'-nucleotidase (E.C. 3.1.3.5, CD73) were analyzed in platelets of type 2 diabetic, hypertensive and type 2 diabetic/hypertensive patients. The results showed an increase in platelet **NTPDase** activity in type 2 diabetic (34% and 72%), hypertensive (32% and 70%) and type 2 diabetic/hypertensive patients (30% and 55%) when compared to control

(P<.01) with ATP and ADP as substrate, respectively. 5'-Nucleotidase activity was elevated in the hypertensive (60%) and type 2 diabetic/hypertensive (53%) groups when compared to the control and type 2 diabetic group (P<.01). No differences in sensitivity to inhibitors was detected between the platelets of controls and type 2 diabetic/hypertensive patients. No effects on the enzyme activities were observed when pharmacological doses of propranolol, captopril, furosemide, chlorpropamide, acetylsalicylic acid and glibenclamide were administered. Furthermore, changes in platelet adhesiveness and reactivity were found in all groups tested. In conclusion, we may postulate that **NTPDase** and 5'-nucleotidase from platelets are altered in patients with type 2 diabetes and hypertension. Probably, such alterations are involved in compensatory physiological responses in these diseases and are related to other important mechanisms of thromboregulation.

L13 ANSWER 5 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:386241 The Genuine Article (R) Number: 673YE. Asparagine 81, an invariant glycosylation site near apyrase conserved region 1, is essential for full enzymatic activity of ecto-nucleoside triphosphate diphosphohydrolase 3. Murphy D M; Kirley T L (Reprint). Univ Cincinnati, Coll Med, Dept Pharmacol & Cell Biophys, POB 670575, Cincinnati, OH 45267 USA (Reprint); Univ Cincinnati, Coll Med, Dept Pharmacol & Cell Biophys, Cincinnati, OH 45267 USA. ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS (1 MAY 2003) Vol. 413, No. 1, pp. 107-115. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. ISSN: 0003-9861 . Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB N-linked glycosylation is important for the function, cellular localization, and oligomerization of membrane-bound ecto-nucleoside triphosphate diphosphohydrolases (eNTPDases). NTPDase3 is a prototypical cell membrane-associated eNTPDase, which is equally related and enzymatically intermediate to the other two cell surface membrane **NTPDases** (NTPDasel and 2). The protein sequence of NTPDase3 contains seven putative N-glycosylation sites located in the ecto-domain. Only one of these putative glycosylation sites, asparagine 81 in NTPDase3, which is located near apyrase conserved region 1 (ACR1), is invariant in all the cell surface membrane eNTPDases. Using site-directed mutagenesis, mutants were constructed to eliminate this highly conserved N-glycosylation site in NTPDase3. The results indicate that glycosylation at this position is essential for full enzymatic activity, with mutant ATPase activity decreased more than ADPase activity. Enzymatic deglycosylation of this site is shown to be responsible for the inactivation of the wild-type enzyme by treatment with peptide N-glycosidase-F. In addition, glycosylation of this conserved site is necessary for the stabilization/stimulation of nucleotidase activity upon treatment with the lectin concanavalin A. However, lack of glycosylation at this site did not result in large changes in tertiary or quaternary structure, as measured by Cibacron blue binding, chemical cross-linking, and native gel electrophoretic analysis. Since this N-glycosylation site is invariant in cell membrane eNTPDases, it is postulated that glycosylation of this residue near ACR1 is crucial for full enzymatic activity of the cell membrane **NTPDases**.

L13 ANSWER 6 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:315038 The Genuine Article (R) Number: 662VW. Metabolic control of excessive extracellular nucleotide accumulation by **CD39** /ecto-nucleotidase-1: Implications for ischemic vascular diseases. Marcus A J (Reprint); Broekman M J; Drosopoulos J H F; Islam N; Pinsky D J; Sesti C; Levi R. Columbia Univ, Coll Phys & Surg, Div Cardiol, Vet Affairs New York Harbor Healthcare Syst, 423 E 23rd St, New York, NY 10010 USA (Reprint); Columbia Univ, Coll Phys & Surg, Div Cardiol, Vet Affairs New York Harbor Healthcare Syst, New York, NY 10010 USA; Columbia Univ, Coll Phys & Surg, Dept Med, New York, NY 10010 USA; Cornell Univ, Weill Med Coll, Med Serv Hematol Oncol, Dept Med, New York, NY USA; Cornell Univ,

Weill Med Coll, Med Serv Hematol Oncol, Dept Pathol, New York, NY USA; Cornell Univ, Weill Med Coll, Med Serv Hematol Oncol, Dept Pharmacol, New York, NY USA. JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS (APR 2003) Vol. 305, No. 1, pp. 9-16. Publisher: AMER SOC PHARMACOLOGY EXPERIMENTAL THERAPEUTICS. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA. ISSN: 0022-3565. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Platelets are responsible for maintaining vascular integrity. In thrombocytopenic states, vascular permeability and fragility increase, presumably due to the absence of this platelet function. Chemical or physical injury to a blood vessel induces platelet activation and platelet recruitment. This is beneficial for the arrest of bleeding (hemostasis), but when an atherosclerotic plaque is ulcerated or fissured, it becomes an agonist for vascular occlusion (thrombosis). Experiments in the late 1980s cumulatively indicated that endothelial cell **CD39** - an ecto-ADPase - reduced platelet reactivity to most agonists, even in the absence of prostacyclin or nitric oxide. As discussed herein, **CD39** rapidly and preferentially metabolizes ATP and ADP released from activated platelets to AMP, thereby drastically reducing or even abolishing platelet aggregation and recruitment. Since ADP is the final common agonist for platelet recruitment and thrombus formation, this finding highlights the significance of **CD39**. A recombinant, soluble form of human **CD39**, solCD39, has enzymatic and biological properties identical to the full-length form of the molecule and strongly inhibits human platelet aggregation induced by ADP, collagen, arachidonate, or TRAP (thrombin receptor agonist peptide). In sympathetic nerve endings isolated from guinea pig hearts, where neuronal ATP enhances norepinephrine exocytosis, solCD39 markedly attenuated norepinephrine release. This suggests that **NTPDase** (nucleoside triphosphate diphosphohydrolase) could exert a cardioprotective action by reducing ATP-mediated norepinephrine release, thereby offering a novel therapeutic approach to myocardial ischemia and its consequences. In a murine model of stroke, driven by excessive platelet recruitment, solCD39 reduced the sequelae of stroke, without an increase in intracerebral hemorrhage. **CD39** null mice, generated by deletion of apyrase-conserved regions 2 to 4, exhibited a decrease in postischemic perfusion and an increase in cerebral infarct volume when compared with controls. "Reconstitution" of **CD39** null mice with solCD39 reversed these changes. We hypothesize that solCD39 has potential as a novel therapeutic agent for thrombotic diatheses.

L13 ANSWER 7 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2002:307972 The Genuine Article (R) Number: 539JJ. Differential catalytic properties and vascular topography of murine nucleoside triphosphate diphosphohydrolase 1 (NTPDase1) and NTPDase2 have implications for thromboregulation. Sevigny J (Reprint); Sundberg C; Braun N; Guckelberger O; Csizmadia E; Qawi I; Imai M; Zimmermann H; Robson S C. Univ Laval, Ctr Rech Rhumatol & Immunol, CHUQ, 2705 Blvd Laurier, Local T1-49, St Foy, PQ G1V 4G2, Canada (Reprint); Univ Laval, Ctr Rech Rhumatol & Immunol, CHUQ, St Foy, PQ G1V 4G2, Canada; Harvard Univ, Sch Med, Beth Israel Deaconess Med Ctr, Dept Med, Boston, MA 02115 USA; Harvard Univ, Sch Med, Beth Israel Deaconess Med Ctr, Dept Pathol, Boston, MA 02115 USA; Univ Frankfurt, Biozentrum, AK Neurochem, Inst Zool, Frankfurt, Germany. BLOOD (15 APR 2002) Vol. 99, No. 8, pp. 2801-2809. Publisher: AMER SOC HEMATOLOGY. 1900 M STREET. NW SUITE 200, WASHINGTON, DC 20036 USA. ISSN: 0006-4971. Pub. country: Canada; USA; Germany. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Nucleoside triphosphate diphosphohydrolases (**NTPDases**) are a recently described family of ectonucleotidases that differentially hydrolyze the gamma and beta phosphate residues of extracellular nucleotides. Expression of this enzymatic activity has the potential to influence nucleotide P2 receptor signaling within the vasculature. We and others have documented that NTPDase1 (**CD39**, 78 kd) hydrolyzes both triphosphonucleosides and diphosphonucleosides and thereby terminates platelet aggregation responses to adenosine diphosphate (ADP). In

contrast, we now show that NTPDase2 (CD39L1, 75 kd), a preferential nucleoside triphosphatase, activates platelet aggregation by converting adenosine triphosphate (ATP) to ADP, the specific agonist of P2Y(1) and P2Y(12) receptors. We developed specific antibodies to murine NTPDase1 and NTPDase2 and observed that both enzymes are present in the cardiac vasculature; NTPDase1 is expressed by endothelium, endocardium, and to a lesser extent by vascular smooth muscle, while NTPDase2 is associated with the adventitia of muscularized vessels, microvascular pericytes, and other cell populations in the subendocardial space. Moreover, NTPDase2 represents a novel marker for microvascular pericytes. Differential expression of **NTPDases** in the vasculature suggests spatial regulation of nucleotide-mediated signaling. In this context, NTPDase1 should abrogate platelet aggregation and recruitment in intact vessels by the conversion of ADP to adenosine monophosphate, while NTPDase2 expression would promote platelet microthrombus formation at sites of extravasation following vessel injury. Our data suggest that specific **NTPDases**, in tandem with ecto-T-nucleotidase, not only terminate P2 receptor activation and trigger adenosine receptors but may also allow preferential activation of specific subsets of P2 receptors sensitive to ADP (eg, P2Y(1), P2Y(3), P2Y(12)) and uridine diphosphate (P2Y(6)). (C)D 2002 by The American Society of Hematology.

L13 ANSWER 8 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2002:414087 The Genuine Article (R) Number: 549HH. Purification, characterization, cloning, and expression of the chicken liver ecto-**ATP-diphosphohydrolase**. Knowles A F (Reprint); Nagy A K; Strobel R S; Wu-Weis M. San Diego State Univ, Dept Chem, San Diego, CA 92182 USA (Reprint); W Los Angeles Vet Affairs Med Ctr, Los Angeles, CA 90073 USA; Metropolitan State Univ, Dept Nat Sci, St Paul, MN USA. EUROPEAN JOURNAL OF BIOCHEMISTRY (MAY 2002) Vol. 269, No. 9, pp. 2373-2382 . Publisher: BLACKWELL PUBLISHING LTD. P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND. ISSN: 0014-2956. Pub. country: USA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB We previously demonstrated that the major ecto-nucleoside triphosphate phosphohydrolase in the chicken liver membranes is an ecto-**ATP-diphosphohydrolase** (ecto-ATPDase) [Caldwell, C., Davis, M.D. & Knowles, A.F. (1999) Arch. Biochem. Biophys . 362 , 46-58]. Enzymatic properties of the liver membrane ecto-ATPDase are similar to those of the chicken oviduct ecto-ATPDase that we have previously purified and cloned. Using antibody developed against the latter, we have purified the chicken liver ecto-ATPDase to homogeneity. The purified enzyme is a glycoprotein with a molecular mass of 85 kDa and a specific activity of approximate to 1000 U.mg protein(-1) . Although slightly larger than the 80-kDa oviduct enzyme, the two ecto-ATPDases are nearly identical with respect to their enzymatic properties and mass of the deglycosylated proteins. The primary sequence of the liver ecto-ATPDase deduced from its cDNA obtained by RT-PCR cloning also shows only minor differences from that of the oviduct ecto-ATPDase. Immunochemical staining demonstrates the distribution of the ecto-ATPDase in the bile canaliculi of the chicken liver. HeLa cells transfected with the chicken liver ecto-ATPDase cDNA express an ecto-nucleotidase activity with characteristics similar to the enzyme in its native membranes, most significant of these is stimulation of the ATPDase activity by detergents, which inhibits other members of the ecto-nucleoside triphosphate diphosphohydrolase (E-**NTPDase**) family. The stimulation of the expressed liver ecto-ATPDase by detergents indicates that this property is intrinsic to the enzyme protein, and cannot be attributed to the lipid environment of the native membranes. The molecular identification and expression of a liver ecto-ATPDase, reported here for the first time, will facilitate future investigations into the differences between structure and function of the different E-**NTPDases**, existence of liver ecto-ATPDase isoforms in different species, its alteration in pathogenic conditions, and its physiological function.

L13 ANSWER 9 OF 27 MEDLINE on STN DUPLICATE 3

2002190079 Document Number: 21920555. PubMed ID: 11923085. Identification of two distinct E-**NTPDases** in liver of goldfish (Carassius auratus L.). Alleva K E; Espelt M V; Krumschnabel G; Schwarzbaum P J. (Instituto de Quimica y Fisicoquimica Biologicas, Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires, Junin 956, 1113, Buenos Aires, Argentina.) COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY. PART B, BIOCHEMISTRY AND MOLECULAR BIOLOGY, (2002 Apr) 131 (4) 725-31. Journal code: 9516061. ISSN: 1096-4959. Pub. country: England: United Kingdom. Language: English.

AB We have recently reported the existence of ATPase activity capable of hydrolyzing extracellular ATP and localized at the external cell membrane of goldfish hepatocytes [Am. J. Physiol. (1998) 274 R1031]. In the present study, we investigated whether one or more enzymes of the **ATP diphosphohydrolase** family (called E-**NTPDases**) are responsible for the hydrolysis of extracellular ATP and other nucleotides. Using soluble extracts from goldfish liver, enzyme activity was detected in the presence of ATP (32.1+/-4.0 nmol Pi liberated mg protein(-1) min(-1)), ADP (20.7+/-3.3 nmol Pi liberated mg protein(-1) min(-1)) and UTP (20.7+/-1.2 nmol Pi liberated mg protein(-1) min(-1)). In line with the presence of this hydrolytic activity, liver samples separated by non-denaturing gel electrophoresis and subsequently exposed to either ATP, ADP or UTP yielded a single band with enzyme activity and similar electrophoretic mobility. Subsequent SDS-PAGE electrophoresis of the active bands resulted in the appearance of two protein bands with molecular masses of 70 and 64 kDa. Immunoblotting of soluble extracts and microsomes obtained from goldfish liver, using a monoclonal antibody against **CD39** (a well-known E-**NTPDase**), detected a single 97-kDa protein. The enzyme activity measured in solution and in native gels, together with structural information from denaturing gels plus immunoblots, points to the existence, in goldfish liver, of at least two different E-**NTPDases**.

L13 ANSWER 10 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2002:296418 The Genuine Article (R) Number: 535HH. Localization of NTPDase1/**CD39** in normal and transformed human pancreas. Kittel A (Reprint); Garrido M; Varga G. Hungarian Acad Sci, Inst Expt Med, Dept Pathophysiol, Lab Gastrointestinal Res, POB 67, H-1450 Budapest, Hungary (Reprint); Hungarian Acad Sci, Inst Expt Med, Dept Pathophysiol, Lab Gastrointestinal Res, H-1450 Budapest, Hungary; Univ Pompeu Fabra, Inst Municipal Invest Med, Barcelona, Spain. JOURNAL OF HISTOCHEMISTRY & CYTOCHEMISTRY (APR 2002) Vol. 50, No. 4, pp. 549-555. Publisher: HISTOCHEMICAL SOC INC. UNIV WASHINGTON, DEPT BIOSTRUCTURE, BOX 357420, SEATTLE, WA 98195 USA. ISSN: 0022-1554. Pub. country: Hungary; Spain. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Elevated levels of extracellular ATP have been observed in many tumors. We have localized NTPDase1/**CD39**, one of the principal extracellular nucleotide-hydrolyzing enzymes, in normal and cancerous human pancreas. **NTPDase**/E-ATPDase activity was demonstrated with an enzyme histochemical technique on cryosections of human pancreas. Acinar and duct epithelial cells were devoid of E-ATPDase activity in both normal and transformed tissue. Endothelial cells and smooth muscle around blood vessels and larger ducts showed strong activity. Nerves, connective tissue, and the beta-cells of the islets were also stained. In cancerous tissue this activity was diminished in the smooth muscle around the ducts and was absent from newly formed connective tissue. Immunostaining for **CD39** supported these results but revealed the presence of inactive **CD39** in the duct epithelial cells. We hypothesize that the significantly diminished activity of NTPDase1 in the tissues surrounding the ducts may be associated with the processes that lead to tumor formation in human pancreas.

L13 ANSWER 11 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2002:435437 The Genuine Article (R) Number: 554DY. Purine signaling and potential new therapeutic approach: Possible outcomes of **NTPDase** inhibition. Gendron F P (Reprint); Benrezzak O; Krugh B W; Kong Q;

Weisman G A; Beaudoin A R. Univ Missouri, Dept Biochem, Med Sci Bldg, Room M121, 1 Hosp Dr, Columbia, MO 65212 USA (Reprint); Univ Missouri, Dept Biochem, Columbia, MO 65212 USA; Univ Sherbrooke, Dept Biol, Sherbrooke, PQ J1K 2R1, Canada. CURRENT DRUG TARGETS (JUN 2002) Vol. 3, No. 3, pp. 229-245. Publisher: BENTHAM SCIENCE PUBL LTD. PO BOX 1673, 1200 BR HILVERSUM, NETHERLANDS. ISSN: 1389-4501. Pub. country: USA; Canada. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Interest for extracellular nucleotides has increased since the pioneer work of Burnstock in the early seventies. Research on cellular functions modulated by purines and pyrimidines has led to the identification and characterization of the different components of purine signaling, namely purinoceptors and ecto-nucleotidases. Receptors for tri- and diphosphonucleos ides, known as P2 nucleotide receptors, are designated either P2Y receptors, for those coupled to G-proteins, or P2X for those which are ligand gated-ion channels. Ecto-nucleoside triphosphate diphosphohydrolase (**NTPDase**; EC 3.6.1.5), previously identified as ecto-ATPase, ecto-ATPDase or **CD39**, is now considered as the main ecto-nucleotidase responsible for the sequential hydrolysis of beta and gamma phosphates of tri- and diphosphonucleosides. More recently, research has been focused on the development of specific agonists and antagonists to P2 purinoceptors. The need to develop specific inhibitors for **NTPDase** to understand the role of this enzyme has clearly emerged. This paper covers the development of specific molecules targeting purinergic signaling, more specifically the inhibition of **NTPDase** and their impact on the different physiological systems.

L13 ANSWER 12 OF 27 MEDLINE on STN

2002292106 Document Number: 22028452. PubMed ID: 12031690. **ATP diphosphohydrolase** (**NTPDase** 1) in rat hippocampal slices and effect of glutamate on the enzyme activity in different phases of development. Bruno Alessandra Nejar; Bonan Carla Denise; Wofchuk Susana Tchernin; Sarkis Joao Jose Freitas; Battastini Ana Maria Oliveira. (Departamento de Bioquimica, Instituto de Ciencias Basicas da Saude, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600-ANEXO, 90035-003, RS, Porto Alegre, Brazil.) LIFE SCIENCES, (2002 May 31) 71 (2) 215-25. Journal code: 0375521. ISSN: 0024-3205. Pub. country: England: United Kingdom. Language: English.

AB In the present report we describe an **NTPDase** 1 (**ATP diphosphohydrolase**; ecto-apyrase; EC 3.6.1.5) in rat hippocampal slices. The effect of glutamate on the ATPase and ADPase activities in rat hippocampal slices of different ages was also studied since adenosine, the final product of an enzymatic chain that includes **NTPDase** 1 and 5'-nucleotidase, can act upon A1 receptors in turn decreasing the release of glutamate. Hippocampal slices from 7, 14, 20-23 and 60 day-old rats were prepared and ATPase and ADPase activities were measured. The parallelism of ATPase and ADPase activities in all parameters tested indicated the presence of an **ATP diphosphohydrolase**. In addition, a Chevillard plot indicated that ATP and ADP are hydrolyzed at the same active site on the enzyme. ATPase activity was significantly activated by glutamate in 20-23 and 60 day-old rats, but ADPase activity was not activated. These results could indicate distinct behavior of the ATPase and ADPase activities of **NTPDase** 1 in relation to glutamate or the simultaneous action of the ecto-ATPase. Activation of ATPase activity by glutamate may constitute an important role in this developmental period, possibly protecting against the neurotoxicity induced by ATP, as well as producing high levels of ADP, by increasing adenosine production, a neuroprotective compound.

L13 ANSWER 13 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2002:807370 The Genuine Article (R) Number: 600GK. Roles of Asp54 and Asp213 in Ca2+ utilization by soluble human **CD39**/ecto-nucleotidase. Drosopoulos J H F (Reprint). VA New York Harbor Healthcare Syst, Thrombosis Res Lab, Res Serv, Room 13026W, 423 E 23rd St, New York, NY 10010 USA (Reprint); VA New York Harbor Healthcare Syst, Thrombosis Res

Lab, Res Serv, New York, NY 10010 USA; Cornell Univ, Weill Med Coll, Dept Med, Div Hematol & Med Oncol, New York, NY 10010 USA. ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS (1 OCT 2002) Vol. 406, No. 1, pp. 85-95. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. ISSN: 0003-9861. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Soluble human **CD39** (solCD39) rapidly metabolizes nucleotides, especially ADP released from activated platelets, thereby inhibiting further platelet activation and recruitment. Using alanine substitution mutagenesis, we established a functional role for aspartates D54 and D213 in solCD39. Kinetic analyses of D54A and D213A indicated decreased K(m)s of the mutants, compared to wild type, for the cofactor calcium and for the substrates ADP and ATP. These decreases in calcium and nucleotide affinity of the mutants were accompanied by increases in their rate of catalysis. The decreased affinity of the mutants for calcium was responsible for their diminished ability to reverse platelet aggregation in plasma anticoagulated with citrate, a known calcium chelator. Their ADPase activity in the presence of citrated plasma was also decreased, although this could be overcome with excess calcium. Thus, aspartates 54 and 213 are involved in calcium utilization and potentially involved in cation coordination with substrate in the catalytic pocket of solCD39.

L13 ANSWER 14 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:395879 Document No.: PREV200100395879. Thromboregulation by endothelial cells: Significance for occlusive vascular diseases: Response. Marcus, Aaron J. [Reprint author]; Broekman, M. Johan [Reprint author]; Drosopoulos, Joan H. F. [Reprint author]. Hematology-Oncology, VA-New York Harbor Healthcare System/Weill Medical College of Cornell University, New York, NY, USA. Arteriosclerosis Thrombosis and Vascular Biology, (July, 2001) Vol. 21, No. 7, pp. 1251-1252. print.
ISSN: 1079-5642. Language: English.

L13 ANSWER 15 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:395878 Document No.: PREV200100395878. Thromboregulation by endothelial cells: Significance for occlusive vascular diseases. Robson, Simon C. [Reprint author]. Associate Professor of Medicine, Harvard Medical School, Boston, MA, USA. Arteriosclerosis Thrombosis and Vascular Biology, (July, 2001) Vol. 21, No. 7, pp. 1251. print.
ISSN: 1079-5642. Language: English.

L13 ANSWER 16 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2002:232811 Document No.: PREV200200232811. New developments in anti-platelet therapies: Potential use of **CD39**/vascular **ATP diphosphohydrolase** in thrombotic disorders. Qawi, Imrana [Reprint author]; Robson, Simon C. [Reprint author]. Center for Immunobiology, Beth Israel Deaconess Medical Center, 99 Brookline Ave., Research North, Boston, MA, 02215, USA. Current Drug Targets, (June, 2001) Vol. 2, No. 2, pp. 213-214. print.
ISSN: 1389-4501. Language: English.

L13 ANSWER 17 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:100086 The Genuine Article (R) Number: 394WL. The C-terminal cysteine-rich region dictates specific catalytic properties in chimeras of the ectonucleotidases NTPDase1 and NTPDase2. Heine P; Braun N; Sevigny J; Robson S C; Servos J; Zimmermann H (Reprint). Univ Frankfurt, Biozentrum, AK Neurochem, Inst Zool, Marie Curie Str 9, D-60439 Frankfurt, Germany (Reprint); Univ Frankfurt, Biozentrum, AK Neurochem, Inst Zool, D-60439 Frankfurt, Germany; Harvard Univ, Sch Med, Beth Israel Deaconess Med Ctr, Dept Med, Boston, MA USA. EUROPEAN JOURNAL OF BIOCHEMISTRY (JAN 2001) Vol. 268, No. 2, pp. 364-373. Publisher: BLACKWELL SCIENCE LTD. P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND. ISSN: 0014-2956. Pub. country: Germany; USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Ecto-nucleoside triphosphate diphosphohydrolases (E-**NTPDases**) comprise a novel family of ectonucleotidases that are important in the hydrolysis of extracellular nucleotides. The related NTPDase1 (ecto-apyrase) and NTPDase2 (ecto-ATPase) share a common membrane topography with a transmembrane domain at both the N- and C-terminus, an extensive extracellular loop with five 'apyrase conserved regions' (ACR1 to ACR5), and a cysteine-rich C-terminal region. Whereas NTPDase1 expressed in CHO cells hydrolyzes ATP and ADP equivalently, NTPDase2 has a high preference for the hydrolysis of ATP over ADP. In addition recombinant NTPDase1 hydrolyzes ATP to AMP with the formation of only minor amounts of free ADP. In contrast, ADP appears as the major free product when ATP is hydrolyzed by NTPDase2. In order to determine molecular domains responsible for these differences in catalytic properties, chimeric cDNAs were constructed in which N-terminal sequences of increasing length of NTPDase1 were substituted by the corresponding sequences of NTPDase2 and vice versa. The turnover points were contained within ACR1 to ACR5. Chimeric cDNAs were expressed in CHO cells and surface expression was verified by immunocytochemistry. ATP and ADP hydrolysis rates and ADP and AMP product formation were determined using HPLC. Amino-acid residues between ACR3 and ACR5 and in particular the cysteine-rich region between ACR4 and ACR5 conferred a phenotype to the chimeric enzymes that corresponded to the respective wild-type enzyme. Protein structure rather than the conserved ACRs may be of major relevance for determining differences in the catalytic properties between the related wild-type enzymes.

L13 ANSWER 18 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:673946 The Genuine Article (R) Number: 463FF. Targeting platelet aggregation: **CD39** gene transfer augments nucleoside triphosphate diphosphohydrolase activity in injured rabbit arteries. Gangadharan S P; Imai M; Rhynhart K K; Sevigny J; Robson S C; Conte M S (Reprint). Brigham & Womens Hosp, Div Vasc Surg, 75 Francis St, Boston, MA 02115 USA (Reprint); Brigham & Womens Hosp, Div Vasc Surg, Boston, MA 02115 USA; Harvard Univ, Inst Human Genet, Boston, MA 02115 USA; Beth Israel Deaconess Med Ctr, Dept Med, Boston, MA 02215 USA. SURGERY (AUG 2001) Vol. 130, No. 2, pp. 296-303. Publisher: MOSBY, INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 USA. ISSN: 0039-6060. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background. **CD39**, the major endothelial nucleoside triphosphate diphosphohydrolase (**NTPDase**), plays an important role in local thromboregulation. We hypothesized that balloon injury (BI) leads to an acute reduction in arterial **NTPDase** activity that could be restored by a targeted gene delivery strategy.

Methods. Recombinant adenoviral vectors containing human **CD39** (Ad-**CD39**) or beta -galactosidase (Ad-LacZ) were used. Endothelial (ECs) and smooth muscle cells (SMCs) were infected in vitro and **NTPDase** activity measured. New Zealand white rabbits (N = 28) underwent bilateral iliofemoral artery balloon injury, followed by incubation with Ad-**CD39**, Ad-LacZ, or vehicle. Explanted vessels were analyzed for **NTPDase** activity arid localization of **CD39** expression by immunohistochemistry. Deposition of fluorescent-labeled platelets was studied 3 days after injury and vector treatment.

Results. In vitro, Ad-**CD39** infection resulted in a greater than 40-fold increase in adenosine diphosphatase activity in ECs and a 3-fold increase in SMCs. In vivo, **CD39** transgene expression localized to the luminal aspect of Ad-**CD39**-treated vessels. BI resulted in an acute reduction in vessel wall **NTPDase** activity ($P < .05$). Ad-**CD39** augmented **NTPDase** activity when compared with vehicle or Ad-LacZ ($P < .05$). Platelet deposition on the injured arterial surface was modest arid not different between Ad-**CD39**- and Ad-LacZ-treated vessels.

Conclusions. BI decreases native **NTPDase** activity, which can be augmented by adenovirus-mediated gene transfer of **CD39**.

Further studies are required to determine whether targeted delivery of **CD39** could convey thromboprotective properties to an injured vessel.

L13 ANSWER 19 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:865212 The Genuine Article (R) Number: 484PJ. Modulation of extracellular nucleotide-mediated signaling by **CD39**/nucleoside triphosphate diphosphohydrolase-1. Robson S C (Reprint); Enjyoji K; Goepfert C; Imai M; Kaczmarek E; Lin Y; Sevigny J; Warny M. Harvard Univ, Beth Israel Deaconess Med Ctr, Dept Med, Rm 370, 99 Brookline Ave, Boston, MA 02215 USA (Reprint); Harvard Univ, Beth Israel Deaconess Med Ctr, Dept Med, Boston, MA 02215 USA. DRUG DEVELOPMENT RESEARCH (JUN-JUL 2001) Vol. 53, No. 2-3, pp. 193-207. Publisher: WILEY-LISS. DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA. ISSN: 0272-4391. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Extracellular nucleotide stimulation of purinergic/pyrimidinergic type-2 (P2) receptors are components of platelet, endothelial cell (EC), and leukocyte activation that culminate in vascular thrombosis and inflammation in vivo. **CD39**, the prototype nucleoside triphosphate diphosphohydrolase (or **NTPDase**-1), is highly expressed on quiescent endothelium, monocytes, and activated lymphocytes and therefore could influence these pathways. The potential of **NTPDase**-1 to regulate P2-receptor function in the vasculature has been established by our generation of **cd39**-null mice. These mice exhibit a prothrombotic vascular phenotype ascribed to overexpression of tissue factor by endothelial cells following aberrant P2- (and potentially adenosine 2a/3) receptor activation. Mutant mice also show perturbations in hemostasis, secondary to platelet P2Y1-receptor desensitization. In addition, administration of soluble **NTPDase** and/or induction of **CD39** overexpression by adenoviral vectors consistently result in amelioration of vascular injury in several animal models tested. **CD39** is also the major **NTPDase** expressed by monocyte-macrophages (Mo). Upregulation of tissue factor expression by Mo in vitro and alterations in splenic populations in vivo have been observed in **cd39**-null mice. Paradoxical inhibition of integrin-mediated adhesion and transendothelial migration of **cd39**-null Mo are also related to aberrant P2-receptor activation and have also been observed in vitro and in vivo. Overexpression of **CD39** following infection with recombinant adenoviral vectors also blocks LPS-induced ATP secretion and inhibits IL-1 release in vitro. These studies confirm a role for **CD39** in the differential regulation of P2-receptor activity and function in platelets, vascular, and immune cells. (C) 2001 Wiley-Liss, Inc.

L13 ANSWER 20 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2000:825553 The Genuine Article (R) Number: 368FP. CD39L2, a gene encoding a human nucleoside diphosphatase, predominantly expressed in the heart. Yeung G; Mulero J J; McGowan D W; Bajwa S S; Ford J E (Reprint). HYSEQ INC, IMMUNOL GRP, FUNCT GENOM DEPT, 670 ALMANOR AVE, SUNNYVALE, CA 94086 (Reprint); HYSEQ INC, IMMUNOL GRP, FUNCT GENOM DEPT, SUNNYVALE, CA 94086. BIOCHEMISTRY (24 OCT 2000) Vol. 39, No. 42, pp. 12916-12923. Publisher: AMER CHEMICAL SOC. 1155 16TH ST, NW, WASHINGTON, DC 20036. ISSN: 0006-2960 . Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB E-**NTPDases** are extracellular enzymes that hydrolyze nucleotides. The human E-**NTPDase** gene family currently consists of five reported members (**CD39**, CD39L1; CD39L2, CD39L3, and CD39L4). Both membrane-bound and secreted family members have been predicted by encoded transmembrane and leader peptide motifs. In this report, we demonstrate that the human CD39L2 gene is expressed predominantly in the heart. In situ hybridization results from heart indicate that the CD39L2 message is expressed in muscle and capillary endothelial cells. We also show that the CD39L2 gene encodes an extracellular E-**NTPDase**. Flow cytometric experiments show that

transiently expressed CD39L2 is present on the surface of COS-7 cells. Transfected cells also produce recombinant glycosylated protein in the medium, and this process can be blocked by brefeldin A, an inhibitor of the mammalian secretory pathway. The enzymology of CD39L2 shows characteristic features of a typical E-**NTPDase**, but with a much higher degree of specificity for NDPs over NTPs as enzymatic substrates. The kinetics of the ADPase activity exhibit positive cooperativity. The predominance of CD39L2 expression in the heart supports a functional role in regulating platelet activation and recruitment in this organ.

L13 ANSWER 21 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:36411 The Genuine Article (R) Number: 387FV. Assignment of ecto-nucleoside triphosphate diphosphohydrolase-1/**cd39** expression to microglia and vasculature of the brain. Braun N (Reprint); Sevigny J; Robson S C; Enjyoji K; Guckelberger O; Hammer K; Di Virgilio F; Zimmermann H. Univ Frankfurt, Inst Zool, Biozentrum, Marie Curie Str 9, D-60439 Frankfurt, Germany (Reprint); Univ Frankfurt, Inst Zool, Biozentrum, D-60439 Frankfurt, Germany; Harvard Univ, Sch Med, Beth Israel Deaconess Med Ctr, Dept Med, Boston, MA 02215 USA; Natl Cardiovasc Ctr, Res Inst, Dept Etiol & Pathogenesis, Clin Pathol Lab, Osaka 565, Japan; Univ Ferrara, Dept Expt & Diagnost Med, Ctr Biotechnol, I-44100 Ferrara, Italy. EUROPEAN JOURNAL OF NEUROSCIENCE (DEC 2000) Vol. 12, No. 12, pp. 4357-4366. Publisher: BLACKWELL SCIENCE LTD. P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND. ISSN: 0953-816X. Pub. country: Germany; USA; Japan; Italy. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Extracellular nucleotides are ubiquitous extracellular mediators that interact with and activate nucleotide type 2 (P2) receptors. These receptors initiate a wide variety of signalling pathways that appear important for functional associations between neurons and glial cells and for the regulation of blood flow, haemostatic and inflammatory reactions in the brain. Ectonucleotidases are extracellular nucleotide-metabolizing enzymes that modulate P2 receptor-mediated signalling by the regulated hydrolysis of these agonists. A considerable number of ectoenzyme species with partially overlapping substrate and tissue distributions have been described. Major candidates for expression in the brain are members of the ecto-nucleoside triphosphate diphosphohydrolase (E-**NTPDase** or **CD39**) family. The production of **cd39**(-/-) mice and specific reagents have enabled us to analyse the specific cellular distribution of NTPDase1 (**CD39**), the prototype member of the enzyme family, in the mouse brain. Using monospecific antibodies and enzyme histochemical staining, we have identified NTPDase1 as a major ectonucleotidase associated with both microglia and the endothelial and smooth muscle cells of the vasculature. NTPDase1 is not expressed by neurons and astrocytes. Additional unidentified ectonucleotidase functional activity is observed at lower levels throughout the brain parenchyma. NTPDase1 may regulate P2 receptor-mediated functions of microglia as well as influence nucleotide signalling between neurons or astrocytes that are associated with multiple microglial ramifications. The expression of NTPDase1 by cerebrovascular endothelial and smooth muscle cells also suggests involvement in the regulation of blood flow and thrombogenesis.

L13 ANSWER 22 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:1294 The Genuine Article (R) Number: 383HW. Ca2+-channel blockers and nucleoside triphosphate diphosphohydrolase (**NTPDase**) influence of diltiazem, nifedipine, and verapamil. Gendron F P; Latour J G; Gravel D; Wang Y; Beaudoin A R (Reprint). Univ Sherbrooke, Fac Sci, Dept Biol, 2500 Boul Univ, Sherbrooke, PQ J1K 2R1, Canada (Reprint); Univ Sherbrooke, Fac Sci, Dept Biol, Sherbrooke, PQ J1K 2R1, Canada; Univ Montreal, Fac Med, Dept Pathol & Biol Cellulaire, Montreal, PQ H3C 3J7, Canada; Univ Montreal, Fac Arts & Sci, Dept Chim, Montreal, PQ H3C 3J7, Canada. BIOCHEMICAL PHARMACOLOGY (15 DEC 2000) Vol. 60, No. 12, pp. 1959-1965. Publisher: PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. ISSN: 0006-2952. Pub. country: Canada

. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The nucleoside triphosphate diphosphohydrolases (**NTPDase**; EC 3.6.1.5) are a family of ectonucleotidases associated with vascular endothelial and smooth muscle cells. These ectonucleotidases are involved in the control of vascular tone by regulating the level of circulating ATP. Ca2+-channel blocking agents are currently used for the treatment of hypertension. Considering the external localization of the **NTPDase** catalytic site and its Ca2+. requirement for enzyme activity, a possible interference of calcium antagonists (nifedipine, verapamil-HCl, and diltiazem-HCl and some of its metabolites) could be anticipated. To test that hypothesis, an **NTPDase**-enriched particulate fraction was used. Our results show that verapamil, diltiazem, and its metabolites all produced a concentration-dependent inhibition of **NTPDase**, at concentrations greater or equal to 0.1 mM with verapamil and to 0.5 mM with diltiazem and its metabolites, whereas no significant effect was observed with nifedipine. Kinetic studies, carried out to define the mode of action of these drugs, showed a mixed type of inhibition. Based on their respective K-i values (in parentheses, in mM), inhibitory potencies of these molecules were in the following order: desacetyl-N-desmethyldiltiazem (M-2-HCl; 0.6) > verapamil (0.76) > N-desmethyldiltiazem (M-A; 0.9) > diltiazem (2.4) > desacetyl-O-desmethyldiltiazem (M-4-HCl; 3.5) > desacetyl N,O-desmethyldiltiazem (M-6-HCl; 3.9). Hence, these calcium antagonists can be considered as weak **NTPDase** inhibitors. Moreover, based on these Ki values and the range of concentrations found in the brood, **NTPDase** would not be inhibited significantly in vivo. BIOCHEM PHARMACOL 60;12:1959-1965, 2000. (C) 2000 Elsevier Science Inc.

L13 ANSWER 23 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2000:769153 The Genuine Article (R) Number: 361EV. Recombinant adenoviral mediated **CD39** gene transfer prolongs cardiac xenograft survival. Imai M; Takigami K; Guckelberger O; Kaczmarek E; Csizmadia E; Bach F H; Robson S C (Reprint). HARVARD UNIV, SCH MED, BETH ISRAEL DEACONESS MED CTR, DEPT SURG, 99 BROOKLINE AVE, BOSTON, MA 02215 (Reprint); HARVARD UNIV, SCH MED, BETH ISRAEL DEACONESS MED CTR, DEPT SURG, BOSTON, MA 02215; HARVARD UNIV, SCH MED, BETH ISRAEL DEACONESS MED CTR, DEPT MED, BOSTON, MA 02215; ASAHIKAWA MED COLL, DEPT SURG 2, ASAHIKAWA, HOKKAIDO 078, JAPAN. TRANSPLANTATION (27 SEP 2000) Vol. 70, No. 6, pp. 864-870. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 530 WALNUT ST, PHILADELPHIA, PA 19106-3621. ISSN: 0041-1337. Pub. country: USA; JAPAN. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background. Extracellular ATP and ADP may be important mediators of vascular inflammation and thrombosis. Nucleoside triphosphate diphosphohydrolase (**NTPDase** or **CD39**) is a vascular ectoenzyme that hydrolyses ATP and ADP; however, this activity is lost during reperfusion injury. We show that the supplementation of **NTPDase** activity within xenograft vasculature using **CD39** recombinant adenoviruses (AdCD39) has protective effects in vivo.

Methods. Recombinant adenoviruses containing human **CD39** or P-galactosidase (Adp-gal) encoding genes were constructed. Hartley guinea pig coronary arteries were perfused ex vivo with University of Wisconsin solution containing 10(9) plaque-forming units of the recombinant adenovirus. Infected grafts were then implanted in the abdomen of complement depleted Lewis rats.

Results. **NTPDase** activities decreased in all grafts within the first 24 hr and subsequently recovered only in those hearts infected with AdCD39. Immunohistological examination of AdCD39-infected grafts confirmed successful **CD39** gene transfer into the endocardium and macrovasculature. Expression of **CD39** modestly prolonged graft survival (90.2+/-5.4 hr, mean+/-SD, n=5) when compared with Ad beta-gal-infected grafts (67.4+/-5.4 hr, P<0.005) and perfusion controls (66.4+/-5.2 hr; P<0.005).

Conclusions. Recombinant adenoviral infection can induce expression of **CD39** within cardiac xenografts and provide survival benefits in

vivo. Our data show that ex vivo infection by recombinant adenovirus vectors can result in vascular expression of a potential therapeutic agent.

L13 ANSWER 24 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2000:683420 The Genuine Article (R) Number: 350MB. **CD39** modulates endothelial cell activation and apoptosis. Goepfert C; Imai M; Brouard S; Csizmadia E; Kaczmarek E; Robson S C (Reprint). HARVARD UNIV, BETH ISRAEL DEACONESS MED CTR, SCH MED, DEPT MED, CTR IMMUNOBIOL, RM 370 H, RES N, BOSTON, MA 02215 (Reprint); HARVARD UNIV, BETH ISRAEL DEACONESS MED CTR, SCH MED, DEPT MED, CTR IMMUNOBIOL, BOSTON, MA 02215. MOLECULAR MEDICINE (JUL 2000) Vol. 6, No. 7, pp. 591-603. Publisher: JOHNS HOPKINS UNIV PRESS . JOURNALS PUBLISHING DIVISION, 2715 NORTH CHARLES ST, BALTIMORE, MD 21218-4319. ISSN: 1076-1551. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: **CD39** is the dominant vascular nucleoside triphosphate diphosphohydrolase (**NTPDase**) that exerts major effects on platelet reactivity by the regulated hydrolysis of extracellular adenine nucleotides. The effects of **NTPDases** on endothelial cell (EC) activation and apoptosis remain unexplored.

Material and Methods: Recombinant replication-deficient adenoviruses were constructed with human **CD39** cDNA (rAdCD39) or the bacterial beta-galactosidase (rAd beta gal).

Results: Intact human umbilical vein EC cultures infected with rAdCD39 had substantial and stable increases in **NTPDase** biochemical activity (14.50 +/- 3.50 Pi nmole/well/min), when contrasted with noninfected cells (0.95 +/- 0.002) and rAd beta gal infected cells (1.01+/-0.02; $p<0.005$). Increased **NTPDase** activity efficiently inhibited immediate type 2Y purinergic receptor (P2Y)-mediated EC activation responses viz. von Willebrand factor secretion in response to extracellular ATP. In addition, **CD39** up-regulation blocked ATP-induced translocation of the transcription nuclear factor (NF)-kappa B to the cell nucleus, and abrogated transcription of mRNA encoding E-selectin, and consequent protein synthesis. **CD39** also decreased the extent of apoptosis triggered by putative type-2X purinergic (P2X7) receptors in response to high concentrations of extracellular ATP in vitro.

Conclusion: These properties of **CD39** indicate primary vascular protective effects with potential therapeutic applications.

L13 ANSWER 25 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2000:415400 The Genuine Article (R) Number: 318XV. Identification, characterization, and immunolocalization of a nucleoside triphosphate diphosphohydrolase in pig liver. Leclerc M C; Grondin G; Gendron F P; Sevigny J; Beaudoin A R (Reprint). UNIV SHERBROOKE, FAC SCI, DEPT BIOL, SHERBROOKE, PQ J1K 2R1, CANADA (Reprint); UNIV SHERBROOKE, FAC SCI, DEPT BIOL, SHERBROOKE, PQ J1K 2R1, CANADA; HARVARD UNIV, NEW ENGLAND DEACONESS HOSP, SCH MED, BOSTON, MA 02215. ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS (15 MAY 2000) Vol. 377, No. 2, pp. 372-378. Publisher: ACADEMIC PRESS INC. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0003-9861. Pub. country: CANADA; USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Different isoforms of nucleoside triphosphate diphosphohydrolases (**NTPDases**; EC 3.6.1.5), also identified as **ATP diphosphohydrolases**, have been previously described in mammalian tissues. We report here the biochemical characterization of **NTPDases** in the pig liver. Optimum pH of catalysis is more acidic for this enzyme than for **NTPDases** (neutral or alkaline pH) found in other mammalian tissues. It is less sensitive to bile salts than the bovine spleen **NTPDase**. Calculated K-m values for ATP and ADP (31 and 21 mu M, respectively) are slightly higher than those reported for the latter enzyme. Electrophoretograms of these enzymes also show different migration patterns. Western blots with Ringo, an antibody that recognizes the different isoforms of mammalian **NTPDases**, show a small but reproducible difference in estimated molecular masses (75 kDa for liver vs

78 kDa for spleen **NTPDase**). A second antibody, generated against a different sequence of **NTPDase** I, does not recognize the liver enzyme, thereby indicating some differences in primary structure. Immunolocalization produced a strong signal on hepatocytes, epithelial cells of the bile duct system, and vascular cells. Immunoreactivity was variable among hepatocytes of different lobules and among hepatocytes within a given lobule. In general, those located in the perilobular zone were more reactive than those located in the central zone and in the periphery of the centrolobular vein. (C) 2000 Academic Press.

L13 ANSWER 26 OF 27 CAPLUS COPYRIGHT 2004 ACS on STN
2000:309176 Document No. 133:144366 Thromboregulatory potential of endothelial **CD39**/nucleoside triphosphate diphosphohydrolase: modulation of purinergic signalling in platelets. Robson, Simon C.; Sevigny, Jean; Imai, Masato; Guckelberger, Olaf; Enjyoji, Keiichi (Department of Medicine, Research North, Beth Israel Deaconess Medical Center, Boston, MA, 02215, USA). Emerging Therapeutic Targets, 4(2), 155-171 (English) 2000. CODEN: ETTAF7. ISSN: 1460-0412. Publisher: Ashley Publications.

AB A review with 119 refs. is given. Abnormal platelet reactivity was linked to unstable angina, myocardial infarction, post-angioplasty stenosis, cerebral ischemia, thrombotic stroke, and a variety of inflammatory vascular disorders assocd. with organ or cell transplantation. Drugs that inhibit blood coagulation, promote fibrinolysis or block platelet activation are important pharmacol. agents in these clin. areas. However, current antiplatelet modalities have multiple limitations that preclude widespread and effective therapeutic intervention. Extracellular nucleotide stimulation and purinergic/pyrimidinegic receptor (P2) mediated signalling are key components of platelet and vascular endothelial cell activation responses that culminate in vascular thrombosis. **CD39**, the nucleoside or **ATP diphosphohydrolase** (**NTPDase** or ATPDase) is highly expressed on quiescent endothelium and is the dominant vascular ectonucleotidase hydrolyzing blood plasma ATP and ADP to AMP. The thromboregulatory potential of **CD39** was recently demonstrated by the authors generation of mutant mice with disruption of the gene. These mice exhibit markedly disordered thromboregulation but also show perturbations in hemostasis secondary to platelet P2Y1-receptor desensitization. In addn., the authors have demonstrated in several animal models that administration of sol. **NTPDase** and induction of high level **CD39** expression by adeno-viral vectors consistently results in substantial amelioration of vascular injury. Systemic administration of sol. derivs. of **CD39** or targeted expression of the native protein to sites of arterial injury may have future therapeutic application in vascular diseases.

L13 ANSWER 27 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2000:105846 The Genuine Article (R) Number: 280BA. Modulation of nucleotide triphosphate diphosphohydrolase-1 (**NTPDase**-1)/**cd39** in xenograft rejection. Imai M; Takigami K; Guckelberger O; Enjyoji K; Smith R N; Lin Y; Csizmadia E; Sevigny J; Rosenberg R D; Bach F H; Robson S C (Reprint). HARVARD UNIV, SCH MED, BETH ISRAEL DEACONESS MED CTR, DEPT SURG, 99 BROOKLINE AVE, BOSTON, MA 02215 (Reprint); HARVARD UNIV, SCH MED, BETH ISRAEL DEACONESS MED CTR, DEPT SURG, BOSTON, MA 02215; HARVARD UNIV, SCH MED, BETH ISRAEL DEACONESS MED CTR, DEPT MED, BOSTON, MA 02215; ASAHIKAWA MED COLL, DEPT SURG 2, ASAHIKAWA, HOKKAIDO, JAPAN; MIT, DEPT BIOL, CAMBRIDGE, MA; NATL CARDIOVASC CTR, CLIN PATHOL LAB, OSAKA, JAPAN; MASSACHUSETTS GEN HOSP, DEPT PATHOL, IMMUNOPATHOL UNIT, BOSTON, MA 02114. MOLECULAR MEDICINE (NOV 1999) Vol. 5, No. 11, pp. 743-752. Publisher: SPRINGER VERLAG. 175 FIFTH AVE, NEW YORK, NY 10010. ISSN: 1076-1551. Pub. country: USA; JAPAN. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: There is increasing evidence showing that extracellular nucleotides may be important mediators of vascular inflammation. Nucleotide triphosphate diphos-phohydrolase-1 (**NTPDase**-1, identical to **CD39**), the major vascular endothelial

ectonucleotidase, is responsible for the hydrolysis of both extracellular ATP and ADP in the blood plasma to AMP. Studies were therefore conducted to evaluate the role of vascular **NTPDase-1/cd39** in modulating platelet activation and vascular injury in cardiac xenografts.

Materials and Methods: Cardiac xenografts from both wild-type and **cd39** knockout mice (C57BL/6 x 129 Svj) were transplanted into Lewis rats. Alterations in **cd39** mRNA transcripts and **NTPDase** activity expression were evaluated in wild-type grafts in untreated rats and then following complement depletion and immunosuppression. Rejection responses were studied with both mutant and wildtype grafts in the following models: presensitization with or without complement depletion, complement depletion alone, and with chronic immunosuppression to induce long-term graft survival.

Results: **NTPDase** biochemical activity in wild-type xenografts rapidly decreased after transplantation but soon rebounded with graft survival. Elevated levels of **cd39** mRNA with associated increases in **NTPDase** activity were observed in all long-term surviving wild-type grafts. Hyperacute xenograft rejection times were comparable in wild-type and mutant grafts but **cd39**-deficient grafts were subject to more rapid rejection and exhibited pronounced vascular injury in complement-depleted presensitized rats. The **cd39**-deficient grafts in immunosuppressed recipients were subject to increased intravascular platelet sequestration and fibrin deposition; this resulted in focal myocardial infarction in long-term surviving mutant xenografts.

Conclusions: Augmentation of **NTPDase**-1 activity may be an important adaptive response for graft survival. Our results suggest that **NTPDase**-1/**cd39** influences pathways of vascular injury in cardiac xenografts.

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---Logging off of STN---

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Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	164.21	164.42

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-3.47	-3.47

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